Summer 2004

Expression and Transcriptional Activity of Aryl Hydrocarbon Receptors During Frog Development

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Expression and Transcriptional Activity of Aryl Hydrocarbon Receptors During Frog Development

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Kenyon College Summer Science 2004

Question

•Does dioxin insensitivity and low CYP1A inducibility in embryos result from a lack of AHR *protein* expression?

•Does dioxin insensitivity and low CYP1A inducibility in embryos result from targeted or broad-based repression of AHR signaling activity?

Abstract

Halogenated aromatic hydrocarbons (HAH), including 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD or "dioxin"), cause developmental toxicity in most vertebrates. Several frog species, however, are insensitive to TCDD toxicity, especially during early life stages (Jung 1997, Gutleb 1999). We have used *Xenopus laevis* as a model for studying HAH insensitivity in developing frogs and the underlying molecular mechanisms. Because TCDD toxicity is regulated by the aryl hydrocarbon receptor (AHR), we undertook two studies to determine whether dixoin insensitivity may be due to expression or function of the AHR protein. The data suggest that *Xenopus* are not resistant to TCDD due to a lack of AHR protein, as AHR1α and AHR1β proteins are both detected by Western blotting in early-stage embryos prior to inducibility of CYP1A mRNAs. Differential display experiments indicate a much higher rate of differential transcriptional activity between exposed and unexposed embryos in the later stages than in the early, CYP1A refractory stages. Data from further experiments attempting to verify the differential display results is as yet inconclusive.

Sources and Effects of Dioxins

•Dioxin-like compounds include polychlorinated biphenyls (PCBs), chlorinated dibenzofurans, and chlorinated dibenzodioxins.
•Dioxin-like chemicals are environmental contaminants found in terrestrial, freshwater, and marine environments.
•Released into the environment through industrial processes such as waste incineration and paper milling and as a contaminant of chlorinated herbicids such as Agent Orange.
•Dioxins cause toxic effects in nearly every system of the body; they are hazardous to animals and humans because they bioaccumulate in fatty tissue (Jensen 1987, Poland 1982).
•TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) is the most toxic and best characterized dioxin-like compound.
•Frogs such as *Xenopus laevis* are usually insensitive to the toxic effects of TCDD, especially in early life stages.

Mechanism of Dioxin Toxicity

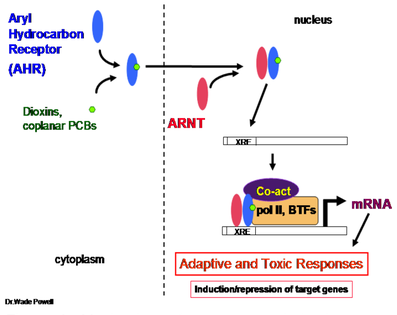

Figure 1: Mechanism of target gene induction or repression by AHR.

•TCDD toxicity is mediated by the aryl hydrocarbon receptor (AHR).

•The AHR is a ligand-activated transcription factor found in mammals, birds, amphibians, and fish that regulates the expression of several genes, some of which may be responsible for toxicity response (Hahn 1998).

•X. laevis expresses two paralogous AHR proteins, AHR1α and AHR1β (Cat Beck, unpublished data).

•TCDD insensitivity in X. laevis may be due to particular functional characteristics of the AHR pathway that differ from other vertebrates.

CYP1A: Biomarker of Dioxin Exposure

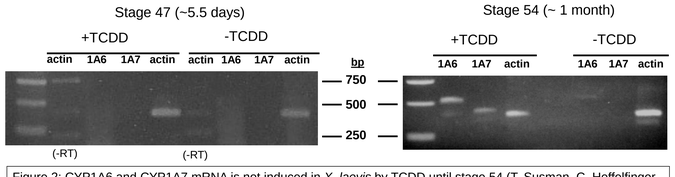

Figure 2: CYP1A6 and CYP1A7 mRNA is not induced in *X. laevis* by TCDD until stage 54 (T. Susman, C. Heffelfinger, N. Chuha, unpublished data).

•CYP1A induction is regulated through the AHR pathway.

•Cytochrome P450 1A genes (CYP1As) are strongly induced by TCDD.

•*X. laevis* have two CYP1A paralogs: CYP1A6 and CYP1A7.

•The lack of CYP1A induction in early life stages may reflect reduced AHR expression or activity.

AHR Protein Expression

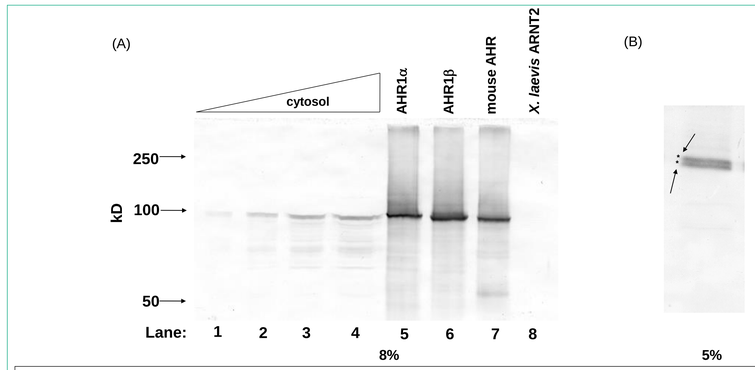

Figure 3: **AHR protein is present in stage 45 *X. laevis* tadpoles.** (**A**) Tadpoles were homogenized and microsomes removed by ultracentrifugation. Cytosol fraction (6 to 92 µg protein) was subjected to 8% SDS-PAGE and Western blotting with an anti-AHR antibody. The cytosol samples (lanes 1-4) contained immunoreactive protein identical in size to synthetic *X. laevis* AHR1α and AHR1β (lanes 5 and 6). Synthetic mouse AHR and *X. laevis* ARNT2 proteins were used as controls (lanes 7 and 8). Positions of molecular weight markers are indicated with arrows, left. (**B**) Separating cytosolic protein by 5% SDS-PAGE permitted resolution of two distinct bands. We suggest these represent AHR1α and AHR1β.

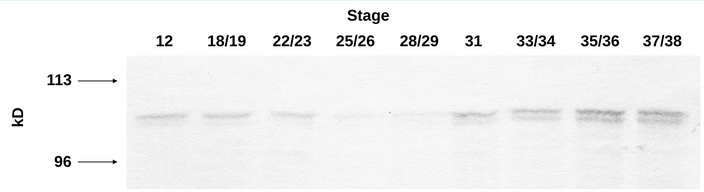

Figure 4: **AHR protein is present from stage 12 in *X. laevis* tadpoles.** Tadpoles were homogenized and cytosol (30 µg protein) was subjected to 5% SDS-PAGE and Western blotting with an anti-AHR antibody. Putative AHR1α and AHR1β proteins are readily visible from stage 31 onwards. Positions of molecular weight markers are indicated with arrows, left.

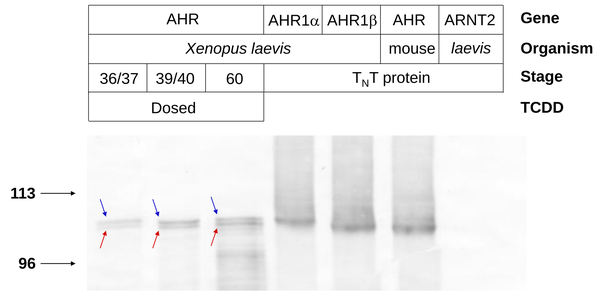

Figure 5: **AHR protein is present in both early- and late-stage embryos after TCDD exposure.** Dosed tadpoles were homogenized and cytosol (15 µg protein) was subjected to 5% SDS-PAGE and Western blotting with an anti-AHR antibody. AHR proteins are present both pre- and post-CYP1A mRNA induction. Putative AHR1α (blue arrows) and AHR1β (red arrows) proteins are readily visible in all protein samples. Positions of molecular weight markers are indicated with arrows, left.

Summary and Conclusions

•Synthetic TNT immunoreactive AHR1α and AHR1β proteins accurately line up with AHR bands from the tadpole cytosol preps (figure 3).

•AHR protein is present in CYP1A-refractory life stages. At least one immunoreactive band is present as early as stage 12. Both putative AHR1α and AHR1β are clearly present from at least stage 31, if not earlier (figure 4).

•AHR protein is also present in TCDD-exposed embryos before and after onset of CYP1A mRNA inducibility (figure 5).

•We suggest that because the two bands in each cytosol sample line up with the bands from the synthesized AHR1α and AHR1β protein, they represent AHR1α and AHR1β expressed by tadpoles.

•Because AHR1α and AHR1β proteins are present in early-stage embryos, regardless of TCDD exposure, the lack of CYP1A inducibility in early-stage *X. laevis* is likely related to AHR function, <u>not</u> AHR expression.

"GeneFishing" for Additional Target Genes

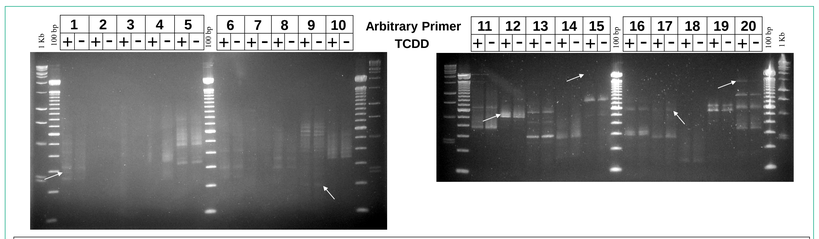

Figure 5: **TCDD-induced differential gene expression in early embryos (stages 33-38)**. Arrows indicate the up-regulated genes in each arbitrary primer (ACP) pair. A total of six differentially expressed genes were identified in early stage embryos. Four were up-regulated and two were downregulated in the presence of TCDD. GeneFishing™ RT-PCR, 3 ng of RNA; 2% agarose gel stained with ethidium bromide.

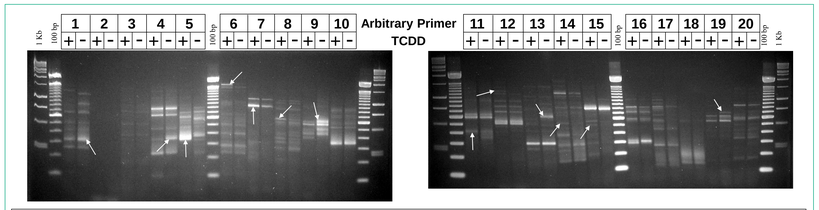

Figure 6: **TCDD-induced differential gene expression in tadpoles (stages 62-64)**. Arrows indicate the up-regulated genes in each arbitrary primer (ACP) pair. A total of thirteen differentially expressed genes were identified in early stage embryos. Eight were up-regulated and five were down-regulated in the presence of TCDD. GeneFishing™ RT-PCR, 3 ng of RNA; 2% agarose gel stained with ethidium bromide.

Summary and Conclusions

•Stage 33-38: TCDD induced differential expression of 6 genes.

•Stage 62-64 embryos: TCDD induced differential expression of 13 genes

Variation in TCDD-induced changes in gene expression between embryos and tadpoles could be due to:

•Reduced AHR transcriptional activity in early-stage embryos.

OR

•greater overall transcriptional activity in later life stages, which are anatomically and physiologically more complex than early embryos. Decreased overall transcription includes a larger number of TCDD-regulated genes.

ID of Cloned Products

From the bands isolated from the late-stage differential display gel, three genes have been identified with high homology to known *Xenopus* genes.

1) **Myosin heavy chain.** Myosin heavy chain is present in smooth muscle, such as that found in the heart (Katz 1992). An upregulation of this gene may indicate an increase in smooth muscle somewhere in the body.

2) **Transgelin 2.** Transgelin is an actin-gelling protein that converts actin filaments, such as those found in muscle, from loose random distribution to discrete tightly bundled foci (Shapland 1993). It is possible that the expression of myosin heavy chain and transgelin 2 may indicate an increase in muscle. Cardiotoxicity in chickens caused by TCDD results in an increase in heart weight (Walker 2000), which may indicate that the same is occuring in exposed late-stage *Xenopus*.

3) **Granulin.** The specific function of granulin has not yet been determined. However, progranulin, granulin's precursor, is expressed in response to endothelial tissue injury (He 2003). TCDD causes endothelial tissue injury in zebrafish (Henry 1997), so it is possible that it may cause the same effect in *Xenopus*, albeit with sublethal effect.

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Acknowledgments

I would like to thank Dr. Wade Powell for his guidance on this project. I would also like to thank Ashley Rowatt, Jeremy Lavine, Aric Whittington, and Blythe Philips. This work was funded by the Kenyon College Summer Science program and the National Institute of Health (R15 ES011130).